maximum pressure of ~60 atm. Two recently discussed transport models, push-and-roll (Yu *et al.* 2010) and one-way-revolution (Zhao *et al*. 2013), propose that the ATPase pushes the DNA directly into the procapsid; additionally, the latter model postulates that the connector acts as a one-way valve and restricts DNA leakage by specific loop interactions (residues N229-N246). Here, we focus on the connector's role in translocation and how it affects DNA conformation. Specifically, how such a one-way valve withstands the large pressure difference and how the connector loop-residues affect the function. To address these questions, we performed equilibrium and force-probe molecular dynamics simulations of the connector with and without DNA. We observe that the connector deforms DNA, which untwists, over-twists and compresses. Remarkably, comparison of the obtained DNA compression with FRET-FCS measurements of the T4 bacteriophage motor (Ray *et al.* 2010), revealed to be common characteristic of the head-tail bacteriophages. Further, the Young's modulus of the connector central region is comparable to that of structural proteins like collagen, and the obtained heterogonous connector stiffness resembles composite materials. These exceptional elastic properties enable the connector to withstand both longitudinal and lateral pressure generated by the packed DNA and the procapsid, respectively. Furthermore, pushing the DNA into the procapsid requires less force than pulling it out. Upon three loop-residue mutations (K234A.K235A.R237A), the required forces for pushing and pulling become similar, which supports the residues' essential role in the one-way-valve function. Our results corroborate the connector's one-way valve function, whereas rotation and/or revolution motions of DNA proposed in both models remain open for future investigation.

330-Pos Board B85

Probing Protein-Protein Interactions in a Single Virus: Application to HIV Integrase Oligomerization

Doortje Borrenberghs, Wannes thys, Susana Rocha, Jonas Demeulemeester, Peter Dedecker, Johan Hofkens, Zeger Debyser, **Jelle Hendrix**.
Katholieke Universiteit Leuven, Leuven, Belgium.

Direct measurement of protein-protein interactions (PPIs) in single viruses is crucial to provide insight into viral biology, replication and pathogenesis in a spatial and time-resolved manner. We report on an imaging method based on Förster resonance energy transfer (FRET) with fluorescent proteins (FPs) to probe the oligomerization of the human immunodeficiency virus (HIV) type 1 integrase enzyme (IN) in viral particles and in infected cells. We performed a detailed characterization of fluorescently labeled viruses at the single-FP level and show that the fluorescent content of particles can be accurately controlled. We demonstrate that measuring FRET with FPs inside single virions is feasible and prove that IN forms oligomers in the virus, as well as in HIV-1 viral complexes inside infected cells. Our methodology can be applied to the study of any protein targeted into viral particles and to measure interactions with host proteins in infected cells.

331-Pos Board B86

Role of M1 Self-Organization in Influenza Virus Assembly: A Combined Rics and AFM Study

Malte Hilsch[1], Nadine Jungnick[2], Christian Sieben[1], Björn Goldenbogen[1], Edda Klipp[1], Andreas Herrmann[1], **Salvatore Chiantia**[1].
[1]Humboldt-Universität zu Berlin, Berlin, Germany, [2]Louisiana State University, Baton Rouge, LA, USA.

The matrix protein (M1) of influenza virus is generally considered to be the key organizer in the budding of new virions from the plasma membrane (PM) of infected cells. In fact, this protein interacts with viral genetic material and envelope proteins, while binding to the inner leaflet of the PM. Its oligomerization plays a pivotal role in viral organization and function. Of interest, the molecular details of M1 oligomerization or its interaction with lipids and other viral proteins are not fully understood.
In order to clarify the role of M1 in influenza virus assembly, we applied a combination of several quantitative microscopy approaches. We first characterized protein multimerization upon interaction with other viral proteins at the PM of living cells, using Number&Brightness (N&B) microscopy. Second, we used controlled biophysical models of the PM (e.g. supported bilayers) to delve into the details of M1-lipid and M1-M1 interaction, using a combination of Raster Image Correlation Spectroscopy (RICS) and Atomic Force Microscopy (AFM). Our results show that M1 oligomer formation is strongly concentration-dependent and does not necessarily require the presence of other viral proteins. Furthermore, we identified several novel lipid binding partners for M1, including phosphatidic acid and phosphatidylinositol phosphates. Finally, we show that specific interaction with the PM does not influence the oligomerization process but rather modulates the overall M1 binding to the membrane.

332-Pos Board B87

Evaluating the Influence of Environment on Virus Capsid Assembly Pathways through Stochastic Simulation

Gregory R. Smith[1], Lu Xie[2], Byoungkoo Lee[3], Russell Schwartz[1].
[1]Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, USA, [2]Computational Biology, Carnegie Mellon University, Pittsburgh, PA, USA, [3]Mathematics, Georgia State University, Atlanta, GA, USA.

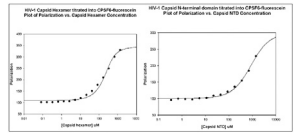
Understanding the unique biochemical and physical differences between typical in vitro experimental systems and the in vivo environment of a living cell is a question of great importance in building and interpreting reliable models of complex reaction systems. Virus capsids make an excellent model system for such questions because they tend to have few components, making them amenable to in vitro and modeling studies, yet their assembly can be described by enormously complex networks of possible reactions that cannot be resolved by any current experimental technology. We have previously attempted to bridge the gap between the complexity of the system and the limitations of data for tracking detailed assembly pathways using simulation-based model inference, learning kinetic parameters of coarse-grained rule models by fitting simulations to light scattering data from in vitro capsid assembly systems. Here, we describe extensions of that work to attempt to understand the influence of specific features of the cellular environment, individually or in concert, on assembly pathway selection. We specifically focus on the effects of macromolecular crowding and nucleic acid on capsid assembly, using coarse-grained biophysical models to adjust rate parameters learned from the in vitro system and suggest how these adjustments to fine-scale interactions may alter high-level pathway selection. Results from a series of virus capsid models suggest surprisingly complex and often counterintuitive mechanisms by which crowding or nucleic acids can alternately promote or inhibit assembly for different virus and assembly conditions.

333-Pos Board B88

Toward Understanding How Cleavage & Polyadenylation Factor 6 Interacts with the HIV-1 Capsid Hexamer

Akash Bhattacharya, Dmitri N. Ivanov.
Biochemistry, UT Health Science Center, San Antonio, San Antonio, TX, USA.

The HIV-1 genetic material enters mammalian cells encapsulated in a shell made of capsid (CA) protein. Cleavage and polyadenylation specificity factor subunit 6 (CPSF6) is a 551AA/68kDa component of the cleavage factor Im complex (CFIm) that plays a key role in pre-mRNA 3'-processing. Researchers have shown that cytosolic CPSF6 (a truncation lacking the C-terminal RS-rich nuclear localization domain) stabilizes the HIV-1 virus core & restricts HIV-1 prior to nuclear import. Mutagenesis studies have identified CPSF6 residues 314 to 322, as being critical for HIV-1 restriction. A recent study has presented a crystal structure of the N-terminal of the HIV-1 capsid (CA-NTD) protein in complex with CPSF6: 313-327.
In our study we present a crystal structure of the polypeptide CPSF6: 313-327 in complex with the complete HIV-1 capsid (CA) hexamer. Titration experiments based on fluorescence polarization anisotropy have been carried out which indicate that fluorescent labeled CPSF6 binds to the hexameric capsid eight times tighter than to the capsid NTD. This, in conjunction with anaytical ultracentrifugation titration experiments provides a greater insight into how CPSF6 binds the viral capsid and causes restriction.



Funding: CPRIT (RP101491)

334-Pos Board B89

Breaking a Virus: Identifying Molecular Level Failure Modes of Viral Capsid Compression through Multi-Scale Simulation Techniques

Venkatramanan Krishnamani[1], Christoph Globisch[2], Christine Peter[2], Markus Deserno[3].
[1]Molecular Physiology and Biophysics, University of Iowa, Iowa City, IA, USA, [2]Department of Chemistry, University of Konstanz, Konstanz, Germany, [3]Department of Physics, Carnegie Mellon University, Pittsburgh, PA, USA.

We use a systematically coarse-grained model for the protein capsid of Cowpea Chlorotic Mottle Virus (CCMV) to study its deformation under uniaxial compression, all the way from its initial elastic response to the capsid's ultimate structural failure. Our model amends the MARTINI force field with an iteratively refined elastic network, and we have previously shown that it reproduces the fluctuations of small fragments as well as the large-scale stress-strain response.

We developed an automated identification method that classifies the contacting protein interfaces in the CCMV capsid into symmetry-classes and characterizes their structural changes upon deformation in residue-level detail. We observed that the symmetry-classes differ markedly in their stability, in a way that appears to backtrack the putative assembly pathway: interfaces that are believed to form last are most likely to break first. For instance, neither protein dimers (the first assembly step) nor pentamers of dimers (the second step) were ever seen to fail, while the hexameric association site (presumably the last to form) ruptures most readily. Interestingly, the wild type capsid fortifies this location with a cooperatively formed 6-stranded beta-barrel motif, which is missing in the mutant we employed in our studies. We hypothesize that interfacial binding strengths regulate the assembly order, but that later (and hence weaker) contacts may be reinforced by cooperative motifs that form post-assembly.

335-Pos Board B90

In Vitro Reconstitution of Membrane Budding by Influenza A Virus Matrix Protein 1

Michael D. Vahey, Daniel A. Fletcher.
Department of Bioengineering, University of California, Berkeley, Berkeley, CA, USA.

Influenza A is an enveloped virus comprised of eight RNA segments that encode the virus's ten major structural and non-structural proteins. In the final stage of its replication cycle, influenza virus's genetic and structural components assemble at the plasma membrane of the infected cell, where they form a bud that extrudes and eventually separates from the host. Although the production of infectious virus particles requires the convergence of all viral components at the site of bud formation, the mechanism by which assembly is coordinated remains poorly understood. One candidate that appears to play a central role is the matrix protein M1. M1 forms an oligomeric layer immediately adjacent to the viral membrane, shaping the virus and providing a link between the envelope proteins (HA, NA, and M2) and the internal ribonucleoprotein (RNP) complex that packages the viral genome. Despite evidence for its importance in the budding process, efforts to characterize the role of M1 and other viral proteins in cells has been challenging due to the complexity of the cellular environment. In order to study influenza A M1 in a well-defined setting, we have developed a minimal in vitro system that reconstitutes M1 assembly and budding on giant unilamellar vesicles. With this system, we have found that M1 oligomerization shapes the membrane into structures that geometrically and topologically resemble the budding virus. These structures are capable of incorporating cargoes with dimensions comparable to the RNP complex, and their assembly drives sorting of both lipids and membrane-bound proteins. By varying the mechanical and biochemical environment in which M1 assembles, we have identified a key mechanistic role for M1 in coordinating influenza A virus bud assembly.

336-Pos Board B91

New Insights on the Versatile Role of the Cholesterol Binding Motif of the HIV-1 Glycoprotein Gp41

Roland Schwarzer[1], Andreas Herrmann[1], Ilya Levental[2], Andrea Gramatica[1].
[1]Molecular Biophysics, Humboldt University, Berlin, Germany, [2]Laboratory of Membrane Biology, Department of Integrative Biology and Pharmacology, The University of Texas, Houston, TX, USA.

Recent experimental results indicate that host cell invasion as well as assembly and budding of the Human Immunodeficiency Virus (HIV) are highly cholesterol dependent. Supposably, cholesterol enriched plasma membrane microdomains, so called rafts, play an important role in different steps of the virus lifecycle. However, the exact function and molecular background of this sensitivity to bilayer compositions remains unknown.

We produced different variants of the HIV transmembrane protein gp41 labeled with a yellow fluorescent protein. Fluorescence lifetime imaging microscopy was used to report Förster Resonance Energy Transfer (FRET) between a raft marker labelled with a cyan fluorescent protein and gp41 chimeras in living cells. Since it is highly distance dependent, occurring FRET reports a co-clustering of both fluorescent protein species in microdomains. By comparison of FRET efficiencies from different truncation and mutation variants of gp41, the Cholesterol Recognition Amino Acid Consensus (CRAC) was identified as main determinant of the protein's raft partitioning in the plasma membrane and interestingly in the Golgi apparatus as well. Moreover, using fluorescence polarization anisotropy microscopy we found indications, that wildtype gp41 oligomers are stabilized in plasma membrane microdomains. However, oligomerization of CRAC mutants was found to be significantly impaired, suggesting a pooling function of the lipid rafts for the assembly and sustainment of functional homo-oligomers. Moreover, flow cytometer experiments revealed a remarkable influence of CRAC mutations on the well-known plasma membrane perturbation properties of gp41. Finally, different biophysical methods were applied to further reveal the role of the cholesterol-CRAC interaction in more details.

Our data provide further insight into the molecular basis and biological implications of the cholesterol dependent lateral protein sorting for the virus assembly processes at cellular membranes.

337-Pos Board B92

The Homopentameric Ring ATPase Motor of the Bacteriophage T4 Tolerates One Inactive Subunit

Vishal I. Kottadiel[1], Li Dai[1], Venigalla B. Rao[1], **Yann R. Chemla**[2].
[1]Biology, The Catholic University of America, Washington, DC, USA, [2]Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Many DNA translocases are multimeric, ringed NTPases. In many cases, how their subunits are coordinated remains poorly understood. Bacteriophage T4 packages its double-stranded DNA genome into a protein capsid using a homopentameric ring of the ATPase gp17. The T4 motor is one of the fastest and most powerful reported to date, translocating DNA at speeds up to 2 kbp/s and capable of packaging against forces greater than 60 pN. Despite extensive biochemical, structural, and single-molecule studies, the mechanism by which the subunits of the T4 motor coordinate to package DNA at high speeds is unknown.

In this work, we present single-molecule investigations of the T4 motor coordination mechanism. We constructed gp17 mutants in which ATPase activity was abolished and fluorescently labeled them with a single Cy3 dye molecule. Mixing labeled mutant with unlabeled wild-type gp17 in different ratios, we assembled ''poisoned'' packaging complexes containing varying numbers of ''dead'' subunits. We used an instrument combining optical tweezers with single-molecule fluorescence microscopy to correlate packaging activity (measured by optical trap) with the number of inactive mutant subunits (measured simultaneously by fluorescence) on each individual complex. Results demonstrate that T4 packaging motors can tolerate a single inactive ATPase in its ring. In contrast to all-wild-type motors, however, packaging in poisoned complexes is interrupted by frequent, transient pauses.

These results suggest that, in contrast to other multimeric motors, the T4 packaging ATPases may not be strictly coordinated. Although a defective subunit may cause packaging to pause, the motor may eventually bypass the impediment. We speculate that this mechanism may allow the T4 motor to attain its high translocation speeds.

338-Pos Board B93

Proton Permeability of HIV Virus like Particles and Vesicular Stomatitis Virus

Pei-I Ku, Jefferey Hodges, Michael L. Landesman, Peter Williams, Xiaolin Tang, Saveez Saffarian.
Physics and Astronomy, University of Utah, Salt Lake City, UT, USA.

Enveloped viruses have been assumed to be impermeable to protons around neutral pH. Here we report the permeability of bald as well as VSV G pseudotyped HIV virus like particles (VLPs) in their mature and immature states as well as wild type VSV virions. Permeability was measured on single isolated virions incorporating pHlorins by changing the external PH from neutral pH to 6.2. pHlorin's fluorescence depends on their protonation state with a pKa of 7.18. We found all HIV VLPs and VSV virions to be permeable to protons, with fast permeation due to nanopore formation at the initial phase followed by the diffusion of protons across the membrane. Maturation of the HIV VLPs increased the permeation rate by a factor of two suggesting the Gag lattice lowers the permeability of immature VLPs. The average permeation rate of the VSV virions as well as HIV VLPs was similar to the previously measured rate for 150 nm lipid vesicles and all virions equilibrated to the outside pH well below 10 min after being subjected to pH 6.2. Our data suggests that permeation of envelopes within VSV virions and HIV VLPs is governed through the same permeation mechanism as lipid vesicles with similar sizes and none of the virions in this study are impermeable, at least if one can wait a sufficiently long time.

339-Pos Board B94

Alix Arrives Late During HIV-1 Assembly

Pei-I Ku, Michael L. Landesman, Saveez Saffarian.
Physics and Astronomy, University of Utah, Salt Lake City, UT, USA.

Human immunodeficiency virus (HIV-1) assembles at the plasma membrane of infected cells and is released after the membrane envelope surrounding the